Quantifying the Distribution of Inhalation Exposure in Human Populations: Distribution of Minute Volumes in Adults and Children

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Assessments of inhalation exposure to environmental agents necessitate quantitative estimates of pulmonary ventilation rates. Estimating a range of exposures in a given population requires an understanding of the variability of ventilation rates in the population. Distributions of ventilation rates (Ve) were described based on the results of a large study where Ve were measured while subjects performed a variety of physical tasks. Three distinct ventilation levels were identified using cluster analyses of the mean Ve and then various activities were assigned to the three levels using a *k*-means procedure. Separate distributions were identified for the three Ve levels for adult males, adult females, and children. The variability of Ve was consistent with a lognormal distribution for all groups. An aggregate daily inhalation rate can be estimated based on the distributions of Ve. *Key words:* exposure, inhalation, minute volume, Monte Carlo, probabilistic, risk assessment. *Environ Health Perspect* 104:974–979 (1996)

Various federal and state programs mandated to protect human health from releases of chemicals into the environment prescribe the performance of a risk assessment to evaluate exposures to chemicals (*1–4*). An essential element of a risk assessment is a quantitative estimate of exposure (*5,6*). Environmental exposures are typically quantified as the product of the concentrations of the toxicants in the media of exposure and the media contact rates normalized to body weight (*7,8*). Other factors that influence exposure, such as the physiochemical characteristics of a toxicant, also need to be addressed.

Default point estimates of media contact rates such as ingestion of 2 liters of water a day (*9*) or the inhalation of 20 m^3 air a day (*10*) have commonly been used to quantify environmental exposures. Given that media contact rates vary among humans, conservative point estimates of media contact have been employed to provide an upper bound estimate of exposure and thereby ensure that exposures are not likely to be underestimated.

Using point estimates for media contact rates in exposure assessments to yield upper bound estimates of exposure does not yield information on how exposure varies among the population at risk. An alternative approach, probabilistic risk assessment, incorporates distributions of media contact rates among human populations to predict ranges or distributions of exposure among a human population. This additional information can be useful in determining how resources should be allocated to address such exposures. However, adequate information concerning the variability of media contact in a human population is essential if the estimated range of exposures is to be meaningful.

Recently, federal legislative initiatives aimed at assessing the human health effects associated with the release of toxicants into the environment have advocated a range of risk estimates as opposed to an upper bound risk estimate (*11–14*). Principles of risk characterization in two proposed statutes include this mandate: "To the extent feasible, the range and distribution of exposures and risks derived from the risk assessment should be included as a component of the risk characterization" (*11,12*). Mandates for a range or a distribution of risk estimates broaden the required assessment of exposure beyond an upper bound point estimate.

Probabilistic risk assessment methods that yield a range of risk, as opposed to a point estimate, incorporate distributions of exposures in a human population into the risk estimates. Investigators have characterized distributions of exposure parameters for drinking-water consumption (*15*), inhalation rate (*16–18*), dietary intake (*19–21*), soil ingestion (*22*), soil dermal adherence (*23*), body weight (*24–28*) and residency duration (*29,30*). Recommendations as to how to employ distributions of these parameters in probabilistic risk assessment are the focus of several reports (*31–35*).

Quantitative estimates of inhalation exposures have been based on ventilation rate and time of exposure valuations. Given that both of these parameters will vary among humans, efforts to ascribe a range or a distribution to inhalation exposure estimates must address the variability of both these parameters. Variation of ventilation rate within the population is probably due to physical activity, age, and gender. These sources of variability will be addressed to establish the variability in ventilation rates within the population.

The results of this study, and of a companion study that segments time spent at various physical activities, will allow for quantitative estimates of the range of inhalation exposures to environmental toxicants. Distributions of ventilation rates, body weight, and time spent in various activities are employed to construct an overall distribution of exposure. Relationships (dependence) between these variables is explored so that an overall joint distribution of inhalation exposure can be developed.

Materials and Methods

California Air Resources Board Study of Ventilation Rates

Analyses were performed on results of a study conducted by the Human Performance Laboratory of the Physical Education Department at the University of California at Davis for the California Air Resources Board (CARB) (*36*). The study's subject population of 160 was selected to approximate California's major ethnic groups (55% Caucasian, 27% Hispanic, 9% Asian, and 8% black). Male and female participants that were representative of four age groups (children between 6 and 12.9 years of age, adolescents between 12 and 18.9 years, adults between 19 and 59.9 years, and adults over 60 years) were recruited from local schools, senior centers, and the university community. Individuals with cardiovascular or pulmonary disease or musculoskeletal impairments were excluded from the study. None of the participants were training for athletic competitions.

Minute volumes (Ve) were measured in each subject during performance of various physical tasks in the laboratory and in the field. Laboratory tasks included resting activities (lying, sitting, and standing) and active activities (walking and running at various speeds on a treadmill). The selection of jogging/running speeds was dependent on the

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subject's age, body weight, and fitness. For resting activities, expired air was collected using a Hans Rudolph two-way breathing valve (model 2700; Hans Rudolph, Kansas City, MO) in a Tissot respirometer for 5 min following a period to acclimate to the posture (15 to 25 min). For active physical tasks, measurements of Ve were obtained using a Parkinson-Cowan Type CD4 high-speed gas meter during the final 3 min of the 6-min time period that the task was performed.

The field activities that were evaluated outside the laboratory were selected to represent normal activities of each age–gender group. Measurements of Ve were obtained following a task familiarization period using portable equipment. Cumulative ventilation volumes were recorded each minute during the field activities in adults and over a 5 min interval in children. Values reported for adults represent 5 min average Ve.

In addition to Ve, measurements taken during each task include heart rate (HR), breathing frequency (f_B), and oxygen consumption (VO_2). Body weight (kg) and height (cm) were recorded. The data were received from CARB on Microsoft Excel spreadsheets (Microsoft Corporation, Redmond, WA) stored on floppy diskettes. The spreadsheets were imported to the statistical software package, Statistica for Windows (StatSoft, Inc., Tulsa, OK) (*37*) with which all statistical analyses were performed.

Establishing Ventilation Groups

Using the age–gender groupings identified in the CARB study, cluster analyses were employed to sort the mean minute ventilation rates for all activities, except adult running, into distinct ventilation groups. Adults and adolescents were combined, while a separate cluster analysis was performed for children.

Euclidean distance

$$(d[x,y] = \sqrt{\sum (x-y)^2})$$

is the measure of the similarity/dissimilarity of the means of the Ve for the various activities. At each step, the closest clusters are joined. Three algorithms, nearest neighbor, farthest neighbor, and paired-group average, were applied to join individual Ve means for the purpose of identifying distinct clusters.

The nearest neighbor algorithm joins the two clusters of Ve means based on the smallest distance between the nearest Ve means. The farthest neighbor algorithm joins clusters according to the farthest distance between Ve means. The unweighted paired-group average uses the average distance between all pairs of Ve means in the two clusters.

Once distinct clusters were identified, the *k*-means procedure was employed to assign individual activities to the various clusters. The advantage of this procedure is that Ve means are moved from cluster to cluster, whereas the joining procedure permanently places Ve means into clusters. Assignments to the clusters were made to minimize the variance within clusters and maximize variance between clusters.

A one-way analysis of variance was employed for each gender at each of the three ventilation levels to identify significant differences of Ve between adults, older adults, and adolescents. In addition, significant differences between males and females and children and adults (adults, older adults, and adolescents) were investigated using a two-way analysis of variance.

Establishing Distributions of Ve

Distributions were established for each ventilation level within each age–gender group in the CARB study. Visual inspection of the histograms of Ve suggested lognormal or gamma distributions. Lognormal and gamma distributions of Ve were assessed using the chi-square goodness-of-fit test and a nonparametric test based on the Kolmogorov-Smirnov test statistic.

Dependent Observations

Since Ve values for multiple activities were obtained from each subject, the observations cannot be considered independent. Therefore, the effect of this dependence on the variance of Ve was evaluated. The impact of dependence upon the magnitude of the variance was measured by comparing the expected value of S_{ve}^2 under the assumption of independent observations versus dependent observations (see Appendix).

Correlation with Body Weight

To use a multivariate exposure model, correlations between the variables must be addressed. Pearson's correlation coefficient, *R*, was used to measure the linear relationship between Ve and body weight.

Results

Establishing Distinct Ve Levels

The physiological changes that take place as one ages are likely to alter the amount of air breathed due to effects on Ve for various activities and differences in the types and amount of physical activity engaged in. Also, physiological differences between males and females may cause disparities in inhalation rates. Hence, the population and sample were segregated into age and gender groups. Cluster analyses were performed on combined adult and adolescent males (≥13 years of age), combined adult and adolescent females (≥13 years of age), and also on combined male and female children (6–12.9 years of age).

Each of the three clustering algorithms yielded dendrograms of the Ve means of the various activities (Fig. 1). For both adult groups, the analysis yielded three distinct clusters of the mean Ve. There was a large Euclidean distance before joining three clusters into two clusters as compared to the distance between joining four clusters into three clusters. Thus, all daily activities of adult males and females were segmented into three ventilation levels.

For children, the farthest neighbor and unweighted paired-group average algorithms yielded three distinct clusters of Ve means. The nearest neighbor algorithm was slow to join walking at greater than 3 mph with running, which suggested four clusters (data not shown). The children's activities were segmented into three ventilation levels, which was supported by two of the three joining algorithms.

Once the three ventilation levels were established, the *k*-means procedure, specifying $k = 3$, was employed to assign the activities reported in the CARB study to the Ve levels (Table 1). Most activities were assigned into the low and moderate levels, but a few activities fell into the high level.

Significant differences between age–gender groups were then identified for each of the three ventilation levels using analyses of variance. Using one-way analyses of variance, mean Ve of adults, older adults, and adolescence males (with the exception of the moderate ventilation level) were not significantly different, or were the mean Ve for the corresponding female groups (p>0.05; data not shown). Therefore, Ve for males 13 years of age and above were pooled and Ve for females age 13 and above were pooled. The mean Ve of male adults was significantly different from the mean Ve for the female group (p<0.05; data not shown). Similarly, mean Ve of male and female children were not significantly different (p>0.05, data not shown). Ve for male and female children were pooled. Three age–gender groups were established for the analysis: adult males aged 13 to 65, adult females aged 13 to 65, and children (males and females) aged 6 to 12.

Distributions of Ve

Separate distributions were established for low, moderate, and high Ve levels for males, females, and children. The histograms of Ve were skewed to the right, suggesting a lognormal or gamma distribution (data not shown). For all distributions, the chi-square goodness-of-fit test did not reject the hypothesis that the ventilation

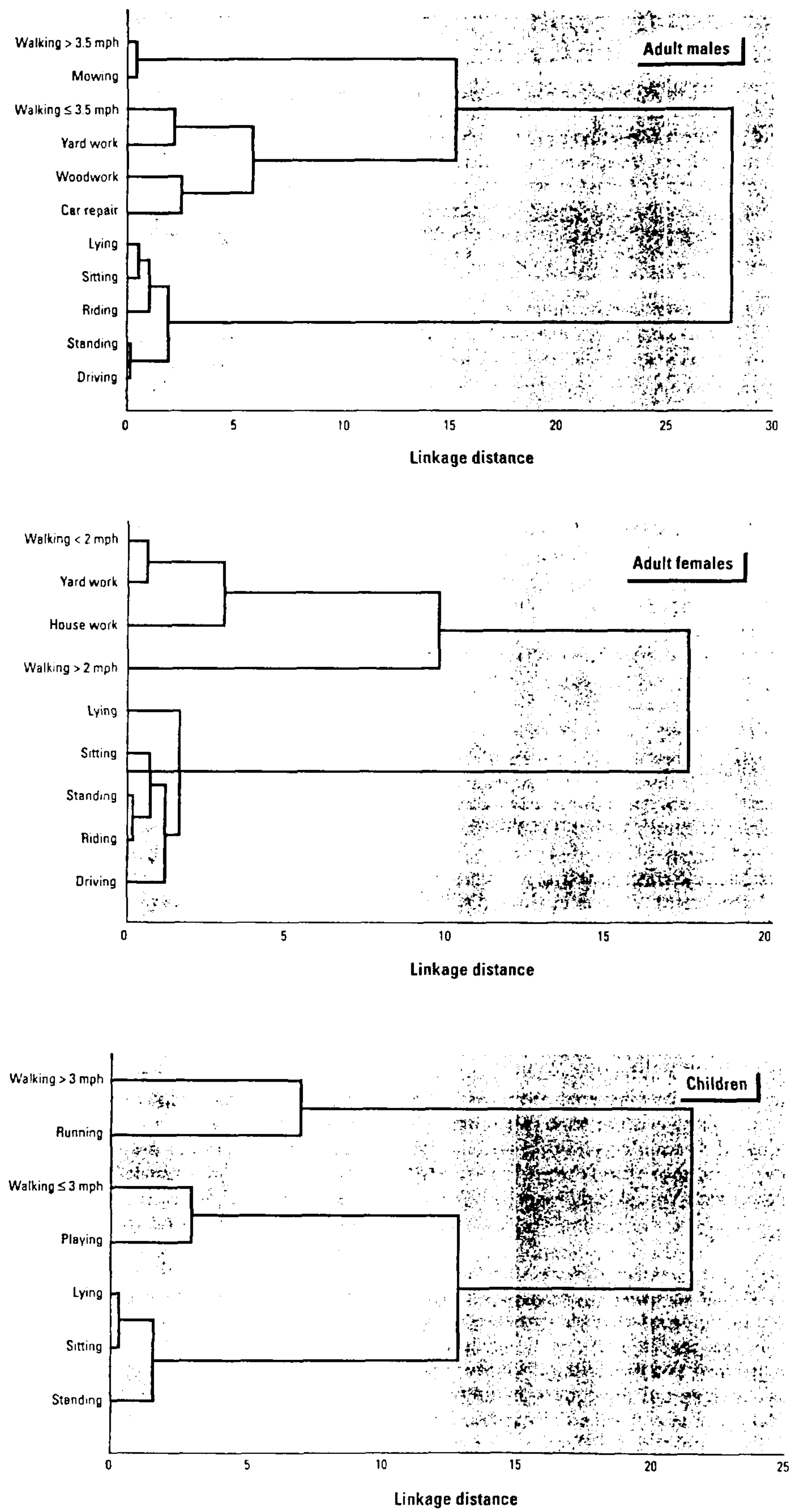

Figure 1. Dendrogram of Ve means of activities of adult males, females, and children using the unweighted paired-group average clustering algorithm.

rates are lognormally distributed (α = 0.05). The Kolmogorov-Smirnov test statistic was not significant for any of the nine cases, also suggesting a lognormal distribution (α = 0.05) (Table 2).

The Ve also fit a gamma distribution for all but two of the nine groups. Overall the chi-square p-values for the lognormal distributions were larger, indicating a better fit to a lognormal distribution.

The mean and standard deviation of Ve and the natural log of Ve for low, moderate, and high Ve levels were determined for males, females, and children (Table 3). Male adults had the highest Ve for all levels, followed by adult females and then children.

Dependent Observations

Ve for numerous activities were obtained from each subject; hence, the observations cannot be considered independent. An analysis of the expected value of the variance of Ve indicated that the dependence of observations reduced the variance of Ve (see Appendix), thus, the variance of Ve for the population is underestimated. The two approaches to eliminating the dependence between multiple observations from a single subject both eliminated components of the total variance of Ve. In light of the deflation of the overall variance due to dependent observations, further decreases in the variance in order to address the dependence is undesirable. Hence, the originally observed Ve were used.

Correlation with Body Weight

Ventilation rate has been correlated with body weight in that ventilation rates increase with increases in body weight (*38*). In this study, Ve was significantly correlated with body weight (Table 4; α = 0.05) and was also correlated with age (Table 5). Thus, correlation coefficients should be used in conjunction with the individual distributions in Monte Carlo simulations to estimate the joint distribution of the correlated variables. In a Monte Carlo simulation, values are selected at random from the distributions, then the values are reordered according to the correlation coefficients. Positive correlation coefficients close to 1.0 indicate strong positive correlation between variables, and negative correlation coefficients close to -1.0 indicate strong negative correlation—highly dependent relationships in either case.

Discussion

Ventilation Levels

Daily inhalation is taken as an aggregation of the product of the amount of air breathed per minute for each activity and the number of minutes per day in the activity. Given that ventilation rates have not been measured for many activities, ventilation levels were determined for clusters of activities. Ventilation levels were established based on the ventilation rates measured in the CARB study of typical human daily activities. Using three joining algorithms, the Ve means of the activities within each age–gender group were joined into three distinct clusters based on a similarity of Ve means. The activities were then assigned to one of the three ventilation levels using the *k*-mean procedure, and an analysis of variance was employed to identify significant differences in Ve means of the various age–gender groups.

All activities in the CARB study except running were used in the analyses for the adult groups. To describe ventilation rates as accurately as possible for all typical activities, ventilation levels should be based on Ve measured during typical human activity. Because adults spend very little time participating in activities with ventilation rates similar to running (*39*), the means of Ve for running were excluded from the list of Ve means for activities in the cluster analysis so as to avoid the undue influence of this activity. Running is a more prevalent activity for children (*40*); therefore, running Ve means are included in the cluster analysis for children.

Measurements of ventilation rates for all physical activities are not available and are not likely ever to be available; however, the grouping of the Ve means from the diverse activities in the CARB study into three very distinct clusters by three different algorithms strongly suggests that most daily activities would fall into one of the three distinct Ve levels. Thus, more comprehensive Ve measurements may not be necessary, given that they are probably adequately represented by the activities included in the CARB study.

The ventilation levels resulting from cluster analysis are low, moderate, and high. Using these groupings, the daily inhalation can be estimated as follows: for a given day, a Ve from the distribution of ventilation rates (liters/minute) for a given Ve level is selected and multiplied by the time spent at that level of activity to obtain the liters of air breathed. Using Monte Carlo or Latin Hypercube procedures, three distributions of inhalation rates are established by selecting values for Ve and minutes in each ventilation level from their respective distributions and then summed to obtain a distribution of daily inhalation:

$$IR = \sum_{\text{Low}}^{\text{High}} (\text{Ve} \times \text{minutes in ventilation level}).$$

Table 1. Assignment of activities to low, moderate and high ventilation levels[a]

Age-gender group	Low	Moderate	High
Adult males	Lying (8.9)[b] Sitting (9.3) Standing (10.6) Riding in a car (9.8) Driving a car (10.7)	Walking 2.5–3 mph (27.4) Woodworking (25.0) Yard work (28.9) Car repair (23.2)	Walking 3.5–4 mph (37.0) Lawn mowing (36.7)
Adult females	Lying (7.1) Sitting (7.7) Standing (8.4) Riding in car (8.2) Driving a car (9.0)	Walking 2–2.8 mph (19.7) Housework (17.4) Yard work (19.3)	Walking 2.8–4 mph (25.8)
Children	Lying (7.5) Sitting (7.3) Standing (8.5)	Walking 2–3 mph (17.8) Playing (17.9)	Walking 3–4 mph (24.1) Running (29.0)

[a]Assignments to the ventilation groups were made using the *k*-means procedure.
[b]Activity and associated mean Ve (l/min) given in parentheses.

Table 2. Chi-square goodness-of-fit test *p*-values and the Kolomogorov-Smirnov test statistics for a lognormal distribution of Ve

Age–gender group	Ve Level	χ^2 *p*-value	K.S. statistic
Adult males	Low	0.64	0.03 (NS)
	Moderate	0.97	0.01 (NS)
	High	0.81	0.02 (NS)
Adult females	Low	0.57	0.04 (NS)
	Moderate	0.62	0.03 (NS)
	High	0.51	0.03 (NS)
Children	Low	0.86	0.02 (NS)
	Moderate	0.18	0.03 (NS)
	High	0.18	0.04 (NS)

The mean Ve were previously reported by CARB (*39*, *40*). NS, not significant.

Table 3. Mean and standard deviation (SD) of Ve and ln(Ve)

Age–gender group	Ve level	Ve[a]	ln(Ve)
Adult males	Low	9.83 ± 2.26	2.09 ± 0.23
	Moderate	26.29 ± 3.27	5.36 ± 0.26
	High	36.44 ± 3.58	8.45 ± 0.23
Adult females	Low	7.92 ± 2.05	1.44 ± 0.23
	Moderate	18.68 ± 2.91	2.63 ± 0.21
	High	25.42 ± 3.23	3.93 ± 0.18
Children	Low	8.02 ± 2.05	1.42 ± 0.21
	Moderate	16.70 ± 2.77	3.50 ± 0.28
	High	28.56 ± 3.32	6.90 ± 0.24

[a]Liters per minute.

Table 4. Correlation coefficients for body weight and ventilation rate

Age–gender group	Activity level	BW	ln(BW)
Adult males	Low ln(Ve)	0.52	0.52
	Mod ln(Ve)	0.72	0.72
	High ln(Ve)	0.73	0.73
Adult females	Low ln(Ve)	0.34	0.35
	Mod ln(Ve)	0.57	0.58
	High ln(Ve)	0.56	0.54
Children	Low ln(Ve)	0.30	0.34
	Mod ln(Ve)	0.68	0.73
	High ln(Ve)	0.70	0.71

Table 5. Correlations between ln(Ve) and age

Age–gender group	Low Ve	Mod Ve	High Ve
Adult males	0.40	0.76	0.61
Adult females	0.11	0.26	0.18
Children	0.23	0.62	0.46

CARB grouped the activities in their study into four categories (light, moderate, heavy and very heavy) of exercise intensity for a 70-kg man based on an Environmental Protection Agency (EPA) criteria document for ozone. Ve were grouped into the EPA categories (for men) or adjusted categories for women and children. The categories are based on overall physiological response to workload. Ve was normalized to body surface area (BSA), based on a correlation between BSA and Ve. For the adult groups, all activities were classed by CARB into the low or moderate categories except running, which was in the heavy category.

In essence, all activities were segregated by CARB into only two of the four Ve categories. In this study, Ve of typical human activities clustered into three distinct ventilation levels based on Ve alone. The low and moderate groups in this study and the CARB study were not equivalent because the activities were grouped differently.

Distributions

Once ventilation levels were established and activities assigned, the distribution of Ve could be determined. Observed Ve during activities from the CARB study provide the basis for the previously mentioned distributional analysis. For each age–gender group, histograms of the observed ventilation rates within each of the Ve levels were skewed to the right. Both lognormal and gamma were considered as a possible distribution for Ve. The chi-square goodness-of-fit test and the nonparametric Kolmogorov-Smirnov test did not reject the null hypothesis that Ve takes on a lognormal distribution. The high p-values for the chi-square goodness-of-fit test and very low Kolmogorov-Smirnov test statistic, which measures the maximum distance from the actual data to the expected lognormal distribution, demonstrate excellent fits. The Ve were also found to fit a gamma distribution in seven of the nine cases, but with weaker overall fits.

Other investigators have suggested distributions for inhalation rate. Brorby and Finley (*16*) present simulated triangular distributions of hourly inhalation rates from adults and children based on a minimum Ve, a maximum Ve, and a most likely Ve with the @RISK simulation software (Palisades Corporation, Newfield, NY) (*16*). Similarly, a uniform distribution of inhalation rates is simulated by Finley and Paustenbach (*17*) using @Risk, based on the minimum and maximum Ve from the EPA study of ventilation rates conducted in 1985. A lognormal distribution of daily inhalation normalized to body weight was proposed based on the assumption that m^3 of air per kilogram of body weight per day is approximated by a lognormal distribution with the mean and standard deviation of Ve designated by the International Commission on Radiological Protection (*18*).

This study employed Ve measured during numerous physical activities to characterize Ve distributions for three activity levels for adult males and females and children. The recent comprehensive CARB study allowed this more thorough analysis than previous efforts. Given the availability of new information in the CARB study, previous estimates of the distribution of Ve based on two or three point estimates and simulations or assumptions are outmoded.

Dependence between Observations

Because Ve for multiple activities within each ventilation level were measured for each individual, the Ve cannot be considered to be independent observations. Two approaches to remove the dependence between the observations were considered (see Appendix). Attempts to remove dependence caused a further undesired reduction in the variance of Ve. Therefore, the observed values of Ve were employed in the analysis.

Correlation with Other Variables in the Exposure Model

The ultimate estimate of exposure generated by Monte Carlo or Latin Hypercube methods is a joint distribution of various exposure parameters. Because a joint distribution must account for correlation between dependent variables, the correlation between Ve and body weight was investigated.

Ventilation rate was correlated with body weight; thus, the correlation coefficient and the distributions for each should be used to establish the joint distribution. Ventilation rates have a lognormal distribution; therefore, the natural log of ventilation rates have a normal distribution. Several investigators have recommended normal or lognormal distributions for body weight (*24–28*). If both factors are normally or lognormally distributed, their bivariate normal distribution can then be determined and accurately represented in Monte Carlo or Latin Hypercube simulations.

Uncertainty

While the CARB study measured Ve for a number of activities, Ve were not ascertained for many physical activities. The use of heart rate (HR) was investigated as a surrogate of Ve. HR is easily measured and has been suggested as a possible predictor of Ve.

The CARB study measured HR and Ve simultaneously, which allowed an evaluation of how well HR predicts Ve using linear regression. In general, HR was found to be poorly correlated to Ve and thus not an appropriate predictor. Another study (*41*) has reached this same conclusion. HR may predict an individual's Ve over different activities but, from person to person, Ve is not adequately predicted by HR.

Three distinct Ve levels were identified from among the mean Ve of the various physical activities, based on cluster analysis. Had Ve been measured for other activities, other Ve levels may have been identified and the assignment of activities to the groups could have changed. Additional measurements of Ve would also be expected to change the described distributions of Ve for the various groups. Measurements were repeated in the same individuals for multiple activities. Independent measurements of activities in separate individuals would be expected to yield more variance than that observed in this study.

The CARB study reported measured Ve in all groups above 6 years of age. Few measurements were obtained for children below the age of 6 years. Therefore, no effort to characterize Ve distributions was attempted for this age group. When further information becomes available, a distribution of Ve for this age group could be described.

Applications

Distributions of ventilation rates were developed that represent the range of everyday human activities of adult males and females and children. In a companion study, distributions of time spent in various activities were established. Using these distributions, a range of inhalation exposures can be quantified using Latin Hypercube or Monte Carlo simulations. Future studies will compare the use of the distributions described in this study with exposure assessments based on upper bound estimates of exposure.

Appendix

Ventilation rates were measured while a number of physical tasks were performed by the same subject. For example, the ventilation rates for standing and lying were measured in subject number 36. Measurements of Ve for different physical activities within an individual would be expected to be more similar than measurements between individuals. Thus, dependent observations would be expected to decrease the variability of the measured Ve. Independent observations are assumed for chi-square goodness-of-fit test, t-tests, and analysis of variance. The variance of the ventilation rates is given by:

$$S_{ve}^2 = \frac{\sum_{i=1}^{n}\sum_{j=1}^{a}(x_{ij} - \bar{x}..)^2}{n-1}$$

where n = number of subjects, a = number of activities, $\bar{x}..$ = grand mean over all subjects and all activities, x_{ij} = observed Ve for subject i, activity j, and $\bar{x}_{i}$ = mean Ve for subject i across all activities and activity-level groups.

Consider the numerator of the variance in the case of two activities. The overall mean is written as the mean of the activity means,

$$\bar{x}.. = \frac{\bar{x}_{.1} + \bar{x}_{.2}}{2}.$$

$$\sum_{i=1}^{n}\left(x_{i1} - \frac{\bar{x}_{.1} + \bar{x}_{.2}}{2}\right)^2 + \sum_{i=1}^{n}\left(x_{i2} - \frac{\bar{x}_{.1} + \bar{x}_{.2}}{2}\right)^2$$
$$= \sum_{i=1}^{n}\left[\left(x_{i1} - \bar{x}_{.1}\right) + \left(\frac{\bar{x}_{.1} - \bar{x}_{.2}}{2}\right)\right]^2$$
$$+ \sum_{i=1}^{n}\left[\left(x_{i2} - \bar{x}_{.2}\right) + \left(\frac{\bar{x}_{.1} - \bar{x}_{.2}}{2}\right)\right]^2$$
$$= \sum_{i=1}^{n}\left(x_{i1} - \bar{x}_{.1}\right)^2 + \frac{n}{2}\left(\bar{x}_{.1} - \bar{x}_{.2}\right)^2$$
$$+ \sum_{i=1}^{n}\left(x_{i2} - \bar{x}_{.2}\right)^2$$

The expected value of this sum of squares provides a gauge of the effect of dependent versus independent observations. The expected value of the first and third terms is unchanged; thus, consider the expected value of the term containing the means over both activities.

$$E\left[\frac{n}{2}\left(\bar{x}_{.1}-\bar{x}_{.2}\right)^2\right]=E\left[\bar{x}_{.1}^{\;2}-2\bar{x}_{.1}\bar{x}_{.2}+\bar{x}_{.2}^{\;2}\right]$$
$$=\mathrm{Var}\left[\bar{x}_{.1}\right]+E\left[\bar{x}_{.1}\right]^2+\mathrm{Var}\left[\bar{x}_{.2}\right]+E\left[\bar{x}_{.2}\right]^2$$
$$-2\left\{\mathrm{Cov}\left[\bar{x}_{.1},\bar{x}_{.2}\right]+E\left[\bar{x}_{.1}\right]E\left[\bar{x}_{.2}\right]\right\}$$

The covariance term equals zero under the assumption of independent observations; otherwise, the term is positive or negative. The sign of the covariance is the same as the sign of the correlation coefficient. In this analysis, the correlation and covariance are positive; thus, the overall variance is decreased by dependence of observations.

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